Synopsis on

ROLE OF LIRAGLUTIDE ALONG WITH FLAVONOIDS IN DIABETIC CARDIOMYOPATHY IN RAT: POSSIBLE MECHANISM OF eNOS/ mPTP/TGF- β

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BY

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SYNOPSIS

Area of specialization: Pharmaceutical Sciences (Pharmacology).

Objective: The present study has been design to investigate Role of liraglutide along with flavonoids in diabetic cardiomyopathy in rat: Possible mechanism of eNOS/ mPTP/TGF-β.

Introduction

By the end of 2030, 79.4 million people in India are anticipated to have type 2 diabetes (T2DM). As a result, immediate action is necessary. A glucagon-like peptide-1 agonist called liraglutide was given US FDA approval for T2DM in 2010 [1]. Without resulting in hypoglycemia, it lowers blood glucose and glycated haemoglobin levels. Once daily subcutaneous administration of commercially available Liraglutide enhances insulin release while glucagon release is inhibited. However, numerous injections and its slower absorption as a result of heptamer production at the injection site could limit its practical applicability. As a result, it is suggested that an oral version of liraglutide be developed. Liraglutide, on the other hand, is a peptide and analogue of endogenous GLP-1 that is digested by the liver and is destroyed enzymatically when taken orally [2].

Hyperglycemia brought on by impaired insulin production, impaired insulin action, or both, is a hallmark of diabetes mellitus, a metabolic illness. Diabetes-related chronic hyperglycemia is linked to microvascular problems that can impair the eyes, kidneys, and nerves in addition to a higher risk of cardiovascular disease. Various anti-diabetic medications are used in the current treatment plans, but these medications have certain drawbacks as well, including hypoglycemia, weight gain, and the short therapeutic half-lives of some medications [3]. In reaction to eating, the gut releases GLP-1, which is made by intestinal L-cells. It affects gastrointestinal motility, causes satiety in the central nervous system, and inhibits glucagon and stomach acid output. Different GLP-1 agonists, including exenatide, liraglutide, dulaglutide, and albuglutide, have been introduced as a novel treatment for type 2 diabetes. GLP-1 agonists are superior to other medications in a number of ways, including the reduction of glycated haemoglobin, control of body weight, preservation of beta cell function, reduction of hyperlipidemia, and reduction of

blood pressure. They imitate the effects of the naturally occurring hormone GLP-1, which is released from the intestine in response to meal intake [4].

Type 2 diabetes has been successfully treated with GLP-1 and GLP-1 agonists. The preparation, characterisation, release studies, and evaluation of the GLP-1 loaded polylactide-co-glycolide (PLGA) microspheres' bioactive investigations are planned. D-ala-GLP-1, a GLP-1 analogue resistant to DPP-4, has been shown to be successfully delivered when administered orally when it is enclosed in PLGA nanoparticles. The GLP-1 agonist that has been the subject of the greatest research is exenatide. Alginate and hyaluronic acid will be cross-linked to create the exanatide-loaded microspheres, which will be administered orally to treat T2DM. Exanatide is a potent insulin-tropic substance, and in order to target the goblet cells, its ability to reduce blood sugar when administered orally will be investigated. Exanatide multilayer nanoparticles' potential for protection in the treatment of T2DM has been evaluated. It has also been assessed how chitosan affects the physiological characteristics of exanatide-loaded PLGA nanoparticles. For the sustained delivery of exanatide to treat T2DM, it has been demonstrated that polymer combination in aqueous medium or sol-gel transition of nanoparticles will be explored [5]. It has been demonstrated that type 2 diabetes can be successfully treated with GLP-1 agonists. Victoza®, a brand name for the commercially available liraglutide, is administered subcutaneously. Liraglutide has not yet been studied via the oral route, which is the preferred option. To the best of our knowledge, there are papers that looked at the impact of using liraglutide along with oral anti-diabetic medications.

Liraglutide is a 3.75 kDa peptide made up of 30 amino acids. With the addition of a glutamic acid residue at position 26, ligarglutide exhibits 97% sequence homology with GLP-1 and allows the attachment of a palmitoyl group. Because it is 97% homologous with native GLP-1, it does not create antibodies. With a longer half-life of 12 to 14 hours following subcutaneous injection and noncovalent albumin binding made easier by the palmitoyl group, it is suited for once-daily dosing. Compared to other anti-diabetic medications, liraglutide has reportedly been shown to improve glycaemic management. With the exception of mild to moderate gastrointestinal side effects, which got better when treatment continued and a very low risk of hypoglycemia, liraglutide is well tolerated. It exhibits more effectiveness than other incretin-based treatments. The numerous injections under the skin in its therapeutic uses could be a drawback. Liraglutide

has a delayed absorption rate following subcutaneous injection because it creates self-associated heptamers there. Subcutaneous injections may hurt the patient and increase their risk of developing an infection at the injection sites [6].

Natural remedies could open up fresh possibilities for cutting-edge treatments meant to stop the progression or emergence of diabetes problems. Galangin, or Gal (Gal; 3,5,7-trihydroxyflavone), is a naturally occurring flavonoid that is present in lesser galangal and honey. It has a variety of biological properties, including antioxidant and anti-inflammatory properties. Gal will be found to reduce apoptosis and restore antioxidant defence mechanisms in rats, preventing ischemia-reperfusion (I/R)-induced hepatic damage. Gal will also be demonstrated to suppress hepatic stellate cell activation and proliferation in addition to protecting rats' livers from CCl4-induced fibrosis and damage [7]. Gal also showed anti-inflammatory action on lipopolysaccharide (LPS)-activated macrophages by reducing the expression of pro-inflammatory genes such as ERK and NF-B p65. Gal has also been discovered to decrease NF-B, which reduces ovalbumin-induced airway inflammation. Gal also reduced inflammatory responses and apoptosis, which protected against pressure overload-induced heart remodelling in vivo and in vitro [8]. Recently, we published research showing that Gal has protective effects against cyclophosphamide (CP)-induced oxidative stress, DNA damage, inflammation, and liver cell death in rats via activation of Nrf2/heme oxygenase-1 (HO-1) signalling [8]. Gal has strong pharmacological effects, however it is still unknown if it can prevent heart damage brought on by diabetes. Therefore, in a rat model of type 1 diabetes, the current work investigated the ameliorative effect of Gal on myocardial oxidative stress, inflammation, and apoptosis. The basic characteristics of cardiac dysfunction, such as altered systolic and diastolic functions, local inflammation, oxidative stress, hypertrophy, and cell death processes in the damaged myocardium, have been observed in rodents treated with type 1 diabetes by streptozotocin (STZ). Therefore, STZ-induced mouse diabetes could essentially mimic cardiac abnormalities in T1DM humans [9]. Therefore, there is a need for a novel medication that, by working through a variety of mechanisms, would stop the development and progression of DCM. This medication works in a number of ways, including by lowering oxidative stress, inflammation, fibrosis, and hypoglycemic activity. Cardiomyopathy

causes a rise in TGF- expression and activity. The purpose of the current investigation is to ascertain the impact of liraglutide and flavonoids on rats with experimental diabetic cardiomyopathy [10].

Objective / plan of work

The objectives of the present study has been follows

- Literature review related to test material, procedure and selected model.
- Preparation of experimental protocol.
- Development of selected experimental model.
- Screening of the test material as per the developed experimental model.
- Data collection, compilation and statistical analysis.

Materials and Methods

ANIMAL DETAIL:

In the current investigation, Wistar albino rats of either sex will be employed. The rats will be housed under a 12-hour light/dark cycle in a room with a humidity level of 50–60% and a temperature of 20–22°C. All experimental methodologies and animal handling techniques will be carried out in accordance with CPSCEA guidelines and with institutional animal ethics committee approval.

Study design:

Groups	**Total no of Animals**
Normal	6
Disease control	6
Disease control+ Standard control	6
Disease control+ Liraglutide	6
Disease control+ flavonoid I	6
Disease control+ Liraglutide + flavonoid I	6
Disease control+ flavonoid II	6
Disease control+ Liraglutide + flavonoid II	6

Induction of Diabetic Cardiomyopathy:

The rats will be given an oral high-fat diet (HFD) made up of 365 grammes of powdered normal pellet diet, 310 grammes of lard, 250 grammes of casein, 10 grammes of cholesterol, 60 grammes of vitamin and mineral mix, 3 grammes of dl-methionine, 1 grain of yeast, and 1 grain of sodium chloride for two weeks, after which a single low dose of streptozotocin at a dose of 35 mg/kg will be given intravenously The investigation will include rats whose blood sugar levels were greater than 200 mg/dl after receiving STZ for one week [11,15].

Parameters to be evaluated

A. Anthrometric

I. Body weight

II. Heart Weight

III. Ratio

B. Enzymatic

I. Lactate dehydrogenase (LDH)

II. Creatine kinase (CK-MB)

III. Nitric oxide (NO)

IV. Troponin T/I

V. AST

C. Haemodynamics

D. Histopathology

E. Estimation of mitochondrial function and integrity

F. Cardiac Collagen Content Marker

G. Western Blot Analysis

ANTHROPOMETRIC MEASUREMENTS

All rats (n=6) will have their body weights measured at the beginning of the experiment and again right before the surgery. In grammes, the average body weight is given. The hearts will be removed after surgery and weighed; the mean heart weight is given in grammes. We'll compute the heart weight to body weight ratio [12].

GLYCEMIC STATUS

Post-meal blood glucose (PPBS) and baseline fasting blood glucose (FBS) levels were also monitored. Prior to the measurement day, the rats will be kept fasting for an overnight period. Rats will be confined with their tails exposed using a restrainer under aseptic settings. After that, 70% alcohol will be used to disinfect the distal part of the tail. The tail will be punctured with the lancet when it has dried. A following drop of blood will be used to assess glucose levels using a glucometer strip after the previous drop of blood has been washed off. There will be two readings taken. In overnight starved rats, postprandial blood glucose (PPBS) will be assessed two hours after food consumption, and measurements will be taken in duplicate.

HEMODYNAMIC PARAMETERS

A 50 mg/kg intraperitoneal dosage of sodium pentobarbitone will be used to anaesthetize rats. Tracheostomies will be performed on sedated rats, and the animals will then be intubated using a 14G polythene cannula. A small animal ventilator will be used to do artificial ventilation on rats (Colombus instruments, USA). The chest wall will then be retracted using adjustable retractors following a median sternotomy in order to expose the heart. To measure left ventricular pressure, a 24G stainless steel cannula will be introduced into the LV and coupled to an electronic pressure transducer (Biopac, USA). Using a digital acquisition device (Biopac, USA), all pressure tracings will be digitally recorded and preserved for offline analysis (acqknowledge 4.4 Software). A 20 second cycle will be averaged out to determine all pressures.

Biochemical Parameters:

At the conclusion of the study, the blood will be measured for all biochemical markers before and one week after STZ..

Myocardial enzyme

Blood samples will be obtained through the retro-orbital sinus at the conclusion of the investigation. Centrifugation at 3000 g for 15 minutes will be used to separate the serum, which will then be stored at -20 oC until analysis. Using readily available test kits, the activity of the troponin T/I creatine kinase MB (CK MB), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) will be estimated.

Assessment of nitric oxide (NO) level

The standard protocol's nitrosative stress marker will be used to determine the levels of nitric oxide. These levels are measured in nanomoles of NO/mg protein as its unit of measurement.

Histopathological Examination and Cardiac Collagen Content

In the histology experiment, tissue will typically be fixed using 10% buffered neutral formalin solution. These tissues will be fixed before being embedded in paraffin wax and microtome-cut into transverse midventricular sections with a thickness of around 5 m. These sections will be stained with picro sirius red F3BA for the evaluation of the fibrosis and haematoxylin and eosin for the assessment of necrosis with myocyte size (i.e. collagen content). Using a microscope, photos of histology slides will be obtained.

Assesssment of mitochondrial function and integrity

Isolation of mitochondria from cardiac tissue

After the heart's perfusion in the control group, the mitochondrion will be immediately isolated from the heart in all of the experiment sets. Additionally, the heart will be immediately segregated from the mitochondria. The Lowry method will be used to ascertain the protein composition of the mitochondria of the experimental rat.

Assesssment of mitochondrial function

The quantity of formazan that is produced during the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) will be used to assess the function of mitochondria. At a wavelength of 595 nm, the amount of formazan produced will be measured spectrophotometrically. The amount of formazan produced throughout the experiment will be expressed in milligram per minute / milligram of protein.

Assesssment of mitochondrial integrity

By detecting MMP formation from fluorescent cationic dye, TMRM, in this approach, mitochondrial integrity will be observed. These mitochondrial functions were established using the technique described by Pachauri et al. in 2018.

WESTERN BLOT ANALYSIS

For measuring eNOS, mPTP, TGF-, and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) in cardiac tissue, Western Blot will be used.

According to the directions, protein will be extracted from heart tissue using homogenization in the Total Protein Extraction Kit (Real Gene; Cat. No. 150001). The Bicinchoninic Acid (BCA) protein assay kit will be used to determine the total protein contents in tissue extracts (G-Biosciences). Immunoblotting will be carried out using SDS-Polyacrylamide gel (10% Resolving gel) and Polyvinylidene fluoride (PVDF) membrane. 1X TBST will be used as the washing buffer, and 5% skim milk will be made as the blocking buffer [13,14].

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